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     (FILE 'HOME' ENTERED AT 09:07:14 ON 11 DEC 2002)

     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 09:07:27 ON
     11 DEC 2002
                SEA ENDOGLUCANASE AND (EGZ OR EGY)
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                4   FILE AGRICOLA
                1   FILE BIOBUSINESS
                9   FILE BIOSIS
                3   FILE BIOTECHABS
                3   FILE BIOTECHDS
                9   FILE BIOTECHNO
                6   FILE CABA
               11   FILE CAPLUS
                2   FILE CEABA-VTB
               10   FILE EMBASE
                7   FILE ESBIOBASE
                2   FILE FEDRIP
                4   FILE FSTA
                1   FILE IFIPAT
               11   FILE LIFESCI
                9   FILE MEDLINE
                7   FILE PASCAL
                9   FILE SCISEARCH
                4   FILE TOXCENTER
                3   FILE USPATFULL
                1   FILE WPIDS
                1   FILE WPINDEX
L1               QUE ENDOGLUCANASE AND (EGZ OR EGY)
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     FILE 'CAPLUS, LIFESCI, EMBASE, BIOSIS, BIOTECHNO, MEDLINE, SCISEARCH'
     ENTERED AT 09:09:09 ON 11 DEC 2002
L2            68 S L1
L3             9 S L2 AND SYNERG?
L4             3 DUP REM L3 (6 DUPLICATES REMOVED)
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=> d l4 ibib ab 1-3

L4 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:10662 CAPLUS
DOCUMENT NUMBER: 136:81966
TITLE: **Synergistic** hydrolysis of amorphous cellulose for ethanol saccharification and fermentation by recombinant Klebsiella oxytoca expressing two **endoglucanases** (CelZ and CelY) from Erwinia chrysanthemi
INVENTOR(S): Ingram, Lonnie O.; Zhou, Shengde
PATENT ASSIGNEE(S): University of Florida, USA
SOURCE: PCT Int. Appl., 132 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002000858	A2	20020103	WO 2001-US19690	20010619
WO 2002000858	A3	20020613		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002159990	A1	20021031	US 2001-885297	20010619

PRIORITY APPLN. INFO.: US 2000-214137P P 20000626
US 2000-219913P P 20000721

AB The present invention provides **endoglucanase** activities for carrying out the degrading of a complex sugar and more preferably, the use of **endoglucanase** activities in particular ratios for optimal degrading of a complex sugar. In addn., the invention provides recombinant host cells engineered for optimal expression and secretion of **endoglucanase** activities suitable for degrading complex sugars. Specifically exemplified are recombinant enteric bacteria, Escherichia and Klebsiella, which express an **endoglucanase** under the transcriptional control of a surrogate promoter for optimal expression. In addn., also exemplified is a recombinant enteric bacterium that expresses two different **endoglucanases** celY and celZ, where each is under the transcriptional control of a surrogate promoter for optimal expression in a particular ratio. The invention provides for the further modification of these hosts to include a secretory protein/s that allow for the increased prodn. and/or secretion of the **endoglucanases** from the cell. In a preferred embodiment, the invention provides for the further modification of these hosts to include exogenous ethanologenic genes derived from an efficient ethanol producer, such as Zymomonas mobilis. A deriv. of Klebsiella oxytoca M5A1 contg. chromosomally integrated genes for ethanol prodn. from Zymomonas mobilis (pdc, adhB) and **endoglucanase** genes from Erwinia chrysanthemi (celY, celZ) produced over 20 000 U **endoglucanase** l-1 activity during fermn. In combination with the native ability to metabolize cellobiose and cellotriose, this strain was able to ferment amorphous cellulose to ethanol (58-76% of theor. yield) without the addn. of cellulase enzymes from other organisms. Erwinia chrysanthemi produces a battery of hydrolases and lyases which are very effective in the maceration of plant cell walls. In summary, these results using, e.g., K. oxytoca strain

SZ21, demonstrate an advancement toward the goal of producing sufficient cellulase enzymes for the direct bioconversion of cellobiosides and amorphous cellulose to ethanol without the addn. of supplemental enzymes. **Endoglucanase** levels produced by this strain are over 10-fold over those previously reported for engineered strains of yeast and other bacteria during ethanol fermn. (Brestic-Goachet et al. 1989, Cho et al. 1999, Cho & Yoo 1999, Misawa et al. 1988, Su et al. 1993, Van Rensburg et al. 1996,1998).

L4 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:223076 BIOSIS
DOCUMENT NUMBER: PREV200200223076
TITLE: Fermentation of cellulooligosaccharides and amorphous cellulose to ethanol by cellulolytic deratives of Klebsiella oxytoca P2.
AUTHOR(S): Zhou, S. (1); Ingram, L. O. (1)
CORPORATE SOURCE: (1) University of Florida, Gainesville, FL USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 534. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print. Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001 ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB It is essential to reduce costs associated with saccharification to allow the use of renewable cellulosic biomass as an industrial feedstock for fuels and chemicals. The genetic engineering of cellulolytic strains of ethanologenic microorganisms for the direct microbial conversion of cellulose to ethanol offers one promising approach. By starting with the ethanologenic strain P2 of Klebsiella oxytoca M5A1 with the native ability to metabolize cellobiose, the need for beta-glucosidase was previously eliminated. Recently, two **endoglucanases** encoded by celZ and celY from Erwinia chrysanthemi were found to exhibit **synergy** with carboxymethyl cellulose and acid-swollen cellulose as substrate. Maximum **synergy** (1.8 fold) was achieved by using an activity ratio of **EGZ** to **EGY** similar to that produced in nature by E. chrysanthemi (19:1). After improving their expression by adding the surrogate promoters from Zymomonas mobilis, these two genes were functionally integrated into the chromosome of P2. The resulting cellulolytic strains produced about 20,000 IU/ml of **endoglucanase** (CMCase activity), over half of which was secreted into the medium by adding the out genes from E. chrysanthemi. In this study, we demonstrate that these cellulolytic strains can convert cellooligosaccharides and acid-amorphous cellulose into ethanol without supplemental fungal cellulase.

L4 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2000:722100 CAPLUS
DOCUMENT NUMBER: 133:360384
TITLE: **Synergistic** hydrolysis of carboxymethyl cellulose and acid-swollen cellulose by two **endoglucanases** (CelZ and CelY) from Erwinia chrysanthemi
AUTHOR(S): Zhou, Shengde; Ingram, Lonnie O.
CORPORATE SOURCE: Institute of Food and Agricultural Sciences, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Journal of Bacteriology (2000), 182(20), 5676-5682 CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Erwinia chrysanthemi produces a battery of hydrolases and lyases which are

very effective in the maceration of plant cell walls. Although two **endoglucanases** (CelZ and CelY; formerly **EGZ** and **EGY**) are produced, CelZ represents approx. 95% of the total carboxymethyl cellulase activity. In this study, we have examd. the effectiveness of CelY and CelZ alone and of combinations of both enzymes using CM-cellulose (CMC) and amorphous cellulose (acid-swollen cellulose) as substrates. **Synergy** was obsd. with both substrates. Maximal **synergy** (1.8-fold) was obsd. for combinations contg. primarily CelZ; the ratio of enzyme activities produced was similar to those produced by cultures of E. chrysanthemi. CelY and CelZ were quite different in substrate preference. CelY was unable to hydrolyze sol. cellooligosaccharides (cellotetraose and cellopentaose) but hydrolyzed CMC to fragments averaging 10.7 glucosyl units. In contrast, CelZ readily hydrolyzed cellotetraose, cellopentaose, and amorphous cellulose to produce cellobiose and cellotriose as dominant products. CelZ hydrolyzed CMC to fragments averaging 3.6 glucosyl units. In combination, CelZ and CelY hydrolyzed CMC to products averaging 2.3 glucosyl units. **Synergy** did not require the simultaneous presence of both enzymes. Enzymic modification of the substrate by CelY increased the rate and extent of hydrolysis by CelZ. Full **synergy** was retained by the sequential hydrolysis of CMC, provided CelY was used as the first enzyme. A general mechanism is proposed to explain the **synergy** between these two enzymes based primarily on differences in substrate preference.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences | Cases

Search Results -

Terms	Documents
L3 same (E.coli or Klebsiella)	3

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L4

Refine Search

Recall Text | Clear

Search History

DATE: Tuesday, December 10, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L4	L3 same (E.coli or Klebsiella)	3	L4
L3	L1 same ethanol	212	L3
L2	L1 Same endoglucanase	11	L2
L1	saccharifi$ same ferment$	1180	L1

END OF SEARCH HISTORY

TITLE: Ethanol production from lignocellulose

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

Generate Collection | Print

Terms	Documents
L3 same (E.coli or Klebsiella)	3

Display Format: - Change Format

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Search Results - Record(s) 1 through 11 of 11 returned.

☐ 1. Document ID: US 20020159990 A1

L2: Entry 1 of 11 File: PGPB Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020159990
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020159990 A1

TITLE: Methods and compositions for simultaneous saccharification and fermentation

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ingram, Lonnie O?apos;Neal	Gainesville	FL	US	
Zhou, Shengde	Gainesville	FL	US	

US-CL-CURRENT: 424/94.61; 435/105, 435/161

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6103464 A

L2: Entry 2 of 11 File: USPT Aug 15, 2000

US-PAT-NO: 6103464
DOCUMENT-IDENTIFIER: US 6103464 A

TITLE: Method of detecting DNA encoding a .beta.-glucosidase from a filamentous fungus

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 5997913 A

L2: Entry 3 of 11 File: USPT Dec 7, 1999

US-PAT-NO: 5997913
DOCUMENT-IDENTIFIER: US 5997913 A

TITLE: Method enhancing flavor and aroma in foods by overexpression of .beta.-glucosidase

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 5554520 A

L2: Entry 4 of 11 File: USPT Sep 10, 1996

US-PAT-NO: 5554520
DOCUMENT-IDENTIFIER: US 5554520 A

TITLE: Ethanol production by recombinant hosts

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 5487989 A

L2: Entry 5 of 11 File: USPT Jan 30, 1996

US-PAT-NO: 5487989
DOCUMENT-IDENTIFIER: US 5487989 A

TITLE: Ethanol production by recombinant hosts

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 5424202 A

L2: Entry 6 of 11 File: USPT Jun 13, 1995

US-PAT-NO: 5424202
DOCUMENT-IDENTIFIER: US 5424202 A

TITLE: Ethanol production by recombinant hosts

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 5231017 A

L2: Entry 7 of 11 File: USPT Jul 27, 1993

US-PAT-NO: 5231017
DOCUMENT-IDENTIFIER: US 5231017 A

TITLE: Process for producing ethanol

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 8. Document ID: US 4220721 A

L2: Entry 8 of 11 File: USPT Sep 2, 1980

US-PAT-NO: 4220721
DOCUMENT-IDENTIFIER: US 4220721 A

TITLE: Method for enzyme reutilization

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

☐ 9. Document ID: JP 2002186938 A

L2: Entry 9 of 11 File: JPAB Jul 2, 2002

PUB-NO: JP02002186938A
DOCUMENT-IDENTIFIER: JP 2002186938 A
TITLE: DISPOSAL METHOD OF CELLULOSE-CONTAINING MATERIAL

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 10. Document ID: WO 8501065 A AU 8433199 A CA 1214742 A DE 3481201 G EP 154646 A EP 154646 B JP 61500002 W US 4628029 A ZA 8406663 A

L2: Entry 10 of 11 File: DWPI Mar 14, 1985

DERWENT-ACC-NO: 1985-074548
DERWENT-WEEK: 198512
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Microbial saccharification of cellulosic substrates to glucose - with thermostable cellulase obtd. from microbispora strain

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

☐ 11. Document ID: US 4220721 A BE 882926 A CA 1128884 A DE 2943684 A DK 8001042 A FI 7902997 A FR 2455081 A GB 2047709 A GB 2047709 B IT 1218885 B JP 55144885 A NL 7908082 A NO 7903101 A SE 7907773 A

L2: Entry 11 of 11 File: DWPI Sep 2, 1980

DERWENT-ACC-NO: 1980-67666C
DERWENT-WEEK: 198038
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Reuse of endoglucanase and cellobiohydrolase enzymes - by selective adsorption onto cellulose materials, for simultaneous saccharification fermentations

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments KWIC | Draw Desc | Image

Generate Collection Print

Terms	Documents
L1 Same endoglucanase	11

Display Format: - Change Format

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☐ 1. Document ID: US 20020159990 A1

L4: Entry 1 of 3 File: PGPB Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020159990
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020159990 A1

TITLE: Methods and compositions for simultaneous saccharification and fermentation

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ingram, Lonnie O?apos;Neal	Gainesville	FL	US	
Zhou, Shengde	Gainesville	FL	US	

US-CL-CURRENT: 424/94.61; 435/105, 435/161

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020137154 A1

L4: Entry 2 of 3 File: PGPB Sep 26, 2002

PGPUB-DOCUMENT-NUMBER: 20020137154
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020137154 A1

TITLE: Methods for improving cell growth and alcohol production during fermentation

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ingram, Lonnie O?apos;Neal	Gainesville	FL	US	
Underwood, Stuart A.	Gainesville	FL	US	

US-CL-CURRENT: 435/161; 435/254.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6333181 B1

L4: Entry 3 of 3 File: USPT Dec 25, 2001

US-PAT-NO: 6333181
DOCUMENT-IDENTIFIER: US 6333181 B1

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L4: Entry 3 of 3 File: USPT Dec 25, 2001

DOCUMENT-IDENTIFIER: US 6333181 B1
TITLE: Ethanol production from lignocellulose

Brief Summary Text (13):
Improved methods for enzymatically converting lignocellulose, for example, to ethanol, are desirable. This invention reports the use of ultrasonic treatment in a Simultaneous Saccharification and Fermentation (SSF) process to enhance the ability of cellulase to hydrolyze mixed office waste paper (MOWP), thereby reducing cellulase requirements by 1/3 to 1/2. SSF is a process wherein ethanologenic organisms, such as genetically engineered micro-organisms, such as Escherichia coli KO11 (Ingram et al., 1991) and Klebsiella oxytoca P2 (Ingram et al., 1995), are combined with cellulase enzymes and lignocellulose to produce ethanol. Enzyme cost is a major problem for all SSF processes.

Detailed Description Text (52):
Doran, J. B., H. C. Aldrich, and L. O. Ingram. "Saccharification and fermentation of sugar canebagasse by Klebsiella oxytoca P2 containing chromosomally integrated genes encoding the Zymomonas mobilis ethanol pathway" 1994. Biotechnol. Bioeng. 44:240-247.

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     INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
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     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 13:59:19 ON
     10 DEC 2002
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              11   FILE BIOCOMMERCE
             435   FILE BIOSIS
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            1012   FILE BIOTECHDS
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             363   FILE CEABA-VTB
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               7   FILE CONFSCI
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               3   FILE EMBAL
             162   FILE EMBASE
             136   FILE ESBIOBASE
              21   FILE FEDRIP
              80   FILE FROSTI
             354   FILE FSTA
              29   FILE GENBANK
               1   FILE HEALSAFE
              66   FILE IFIPAT
             268   FILE JICST-EPLUS
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             227   FILE LIFESCI
              65   FILE MEDLINE
              76   FILE NTIS
             446   FILE PASCAL
              44   FILE PROMT
             385   FILE SCISEARCH
             124   FILE TOXCENTER
             494   FILE USPATFULL
               2   FILE USPAT2
             464   FILE WPIDS
             464   FILE WPINDEX
L1              QUE (SACCHARIFICATION AND FERMENTATION)
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     FILE 'CAPLUS, BIOTECHDS, WPIDS, PASCAL, BIOSIS, SCISEARCH, AGRICOLA'
     ENTERED AT 14:01:01 ON 10 DEC 2002
L2           114 S L1 AND ENDOGLUCANASE
L3            51 S L2 AND (E.COLI OR KLEBSIELLA)
L4            48 S L3 AND ETHANOL
L5            18 DUP REM L4 (30 DUPLICATES REMOVED)
L6            25 S L3 AND (CELZ OR CELY)
L7            25 S L6 AND (COLI OR KLEBSIELLA)
L8            48 S L4 AND ETHANOL
L9            25 S L7 AND ETHANOL
L10            9 DUP REM L9 (16 DUPLICATES REMOVED)
L11           53 S CELZ AND CELY
L12           19 DUP REM L11 (34 DUPLICATES REMOVED)
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L12 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 9

ACCESSION NUMBER: 1997:109914 CAPLUS
DOCUMENT NUMBER: 126:222180
TITLE: Synergistic interaction of the Clostridium stercorarium cellulases Avicelase I (**CelZ**) and Avicelase II (**CelY**) in the degradation of microcrystalline cellulose
AUTHOR(S): Riedel, Kathrin; Ritter, Johannes; Bronnenmeier, Karin
CORPORATE SOURCE: Lehrstuhl fuer Mikrobiologie, Technische Universitaet Muenchen, Munchen, 80 290, Germany
SOURCE: FEMS Microbiology Letters (1997), 147(2), 239-244
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Avicelase I and Avicelase II purified from the cellulolytic thermophile Clostridium stercorarium acted in synergism to hydrolyze microcryst. cellulose. The degree of synergism proved to be dependent on the ratio of the two enzymes and on the type of the cellulosic substrate. The activity of the combined enzymes towards Avicel was about double the sum of the individual activities. No synergism was found with amorphous cellulose prepns. It is shown that the simultaneous concerted action of both Avicelases is required to observe synergism. We suggest that synergism results from an exo-exo type cooperativity and present a mechanistic model explaining the synergistic interaction between Avicelase I and Avicelase II.

L12 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:259768 CAPLUS
DOCUMENT NUMBER: 131:70146
TITLE: Intramolecular synergism in natural and engineered endo-exo-1,4-.beta.-glucanase fusion proteins
AUTHOR(S): Riedel, Kathrin; Bronnenmeier, Karin
CORPORATE SOURCE: Institute for Microbiology, Technical University Munich, Munchen, D-80290, Germany
SOURCE: International Conference on Biotechnology in the Pulp and Paper Industry, 7th, Vancouver, B. C., June 16-19, 1998 (1998), Volume C, C75-C78. Canadian Pulp and Paper Association, Technical Section: Montreal, Que. CODEN: 67NEAW
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The endo/exo-1,4-.beta.-glucanase CelA of Anaerocellum thermophilum contains two catalytic domains. The enzyme was purified and the sequence of the celA gene was detd. Sep. cloning and characterization of the domains shows a remarkable cellulolytic activity of both catalytic regions. Nevertheless, the activity of full-length CelA on cryst. substrates is significantly higher than that of a mixt. of the single components. Like the naturally occurring counterpart, we fused the exoglucanase **CelY** and the endoglucanase **CelZ** of Clostridium stercorarium. Compared to a mixt. of the avicelases the cellulolytic activity of the engineered protein is drastically enhanced. This indicates, analogous to CelA, an efficient intramol. synergism of the two catalytic regions.

L12 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 2000:722100 CAPLUS
DOCUMENT NUMBER: 133:360384
TITLE: Synergistic hydrolysis of carboxymethyl cellulose and acid-swollen cellulose by two endoglucanases (**CelZ** and **CelY**) from Erwinia chrysanthemi
AUTHOR(S): Zhou, Shengde; Ingram, Lonnie O.
CORPORATE SOURCE: Institute of Food and Agricultural Sciences, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Journal of Bacteriology (2000), 182(20), 5676-5682
CODEN: JOBAAY; ISSN: 0021-9193 Oct. 2000
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Erwinia chrysanthemi produces a battery of hydrolases and lyases which are very effective in the maceration of plant cell walls. Although two endoglucanases (**CelZ** and **CelY**; formerly EGZ and EGY) are produced, **CelZ** represents approx. 95% of the total carboxymethyl cellulase activity. In this study, we have examd. the effectiveness of **CelY** and **CelZ** alone and of combinations of both enzymes using CM-cellulose (CMC) and amorphous cellulose (acid-swollen cellulose) as substrates. Synergy was obsd. with both substrates. Maximal synergy (1.8-fold) was obsd. for combinations contg. primarily **CelZ**; the ratio of enzyme activities produced was similar to those produced by cultures of E. chrysanthemi. **CelY** and **CelZ** were quite different in substrate preference. **CelY** was unable to hydrolyze sol. cellooligosaccharides (cellotetraose and cellopentaose) but hydrolyzed CMC to fragments averaging 10.7 glucosyl units. In contrast, **CelZ** readily hydrolyzed cellotetraose, cellopentaose, and amorphous cellulose to produce cellobiose and cellotriose as dominant products. **CelZ** hydrolyzed CMC to fragments averaging 3.6 glucosyl units. In combination, **CelZ** and **CelY** hydrolyzed CMC to products averaging 2.3 glucosyl units. Synergy did not require the simultaneous presence of both enzymes. Enzymic modification of the substrate by **CelY** increased the rate and extent of hydrolysis by **CelZ**. Full synergy was retained by the sequential hydrolysis of CMC, provided **CelY** was used as the first enzyme. A general mechanism is proposed to explain the synergy between these two enzymes based primarily on differences in substrate preference.

L10 ANSWER 9 OF 9 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1989-08366 BIOTECHDS
TITLE: Transfer and expression of an Erwinia chrysanthemi cellulase gene in Zymomonas mobilis;
ethanol preparation; gene cloning
AUTHOR: Brestic-Goachet N; Gunasekaran P; Cami B; *Baratti J C
LOCATION: Universite de Provence, Centre National de la Recherche Scientifique, Laboratoire de Chimie Bacterienne, BP 71, 13277 Marseille Cedex 9, France.
SOURCE: J.Gen.Microbiol.; (1989) 135, Pt.4, 893-902
CODEN: JGMIAN
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Zymomonas mobilis ATCC 10998 has a high potential for **ethanol** production, showing higher **ethanol** yield and productivity than yeasts, but has a limited range of utilizable substrates (glucose, fructose and sucrose). With the aim of allowing direct **fermentation** of cellulose, a cellulase (EC-3.2.1.4) gene from Erwinia chrysanthemi encoding **endoglucanase**-Z was subcloned in Escherichia **coli** using broad host range plasmid pGSS33 as vector. Recombinant plasmid pNB20 was transferred into Z. mobilis by mobilization using helper plasmid RP4. Plasmid pNB20 was stably maintained in **E. coli** and Z. mobilis. The cellulase **celZ** gene was expressed efficiently and the expression level was higher in Z. mobilis than in **E. coli**. The specific activity of the enzyme was comparable to that of the parent strain. The proteins produced by Z. mobilis and E. chrysanthemi had identical immunological and electrophoretic properties. Cellulase biosynthesis occurred during exponential growth of Z. mobilis and 35% of the enzyme was released into the medium without detectable cell lysis. The cellulase was located in the periplasmic space in Z. mobilis. (35 ref)

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 5

ACCESSION NUMBER: 1997:446412 CAPLUS
DOCUMENT NUMBER: 127:175452
TITLE: Production of recombinant bacterial **endoglucanase** as a co-product with **ethanol** during **fermentation** using derivatives of Escherichia **coli** KO11
AUTHOR(S): Wood, B. E.; Beall, D. S.; Ingram, L. O.
CORPORATE SOURCE: Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Biotechnology and Bioengineering (1997), 55(3), 547-555
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This study demonstrates a new approach to reduce the amt. of fungal cellulase required for the conversion of cellulose to EtOH. **E. coli** KO11, a biocatalyst developed for the **fermn**. of hemicellulose syrups, was used to produce recombinant **endoglucanase** as a co-product with EtOH. Seven different bacterial genes were expressed from plasmids in KO11. All produced cell-assocd. **endoglucanase** activity. KO11(pLOI1620) contg. Erwinia chrysanthemi **celZ** (EGZ) produced the highest activity, 3200 IU **endoglucanase**/L **fermn**. broth (assayed at pH 5.2 and 35.degree.). Recombinant EGZ was solubilized from harvested cells by treatment with dil. SDS (12.5 mg/mL, 10 min, 50.degree.) and tested in **fermn**. expts. with com. fungal cellulase (5 filter paper units/g cellulose) and purified cellulose (100 g/L). Using **Klebsiella** oxytoca P2 as the biocatalyst, **fermns**. supplemented with EGZ as a detergent-lysate of KO11(pLOI1620) produced 14%-24% more EtOH than control **fermns**. supplemented with a detergent lysate of KO11(pUC18). These results demonstrate that recombinant bacterial **endoglucanase** can function with fungal cellulase to increase EtOH yield during the simultaneous **saccharification** and **fermn**. of cellulose.

L10 ANSWER 7 OF 9 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1999-00655 BIOTECHDS
TITLE: Engineering ethanologenic Escherichia **coli** for cellulose **fermentation**: secretion of Erwinia **endoglucanase** and integration of **Klebsiella** PTS operon for cellobiose utilization; vector plasmid-mediated Zymomonas mobilis pdc and adhB gene and Erwinia chrysanthemi **celZ** **endoglucanase** gene transfer; metabolic engineering (conference abstract)
AUTHOR: Zhou S; Yomano L P; York S W; Ingram L O
CORPORATE SOURCE: Univ.Florida
LOCATION: The University of Florida, Gainesville, FL, USA.
SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1998) 98 Meet., 397
CODEN: 0005P
ISSN: 0067-2777
98th Annual General Meeting of the American Society for Microbiology, Atlanta, GA, USA, 17-21 May, 1998.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Recombinant Escherichia **coli** have previously been genetically engineered to contain Zymomonas mobilis pdc and adhB genes for the conversion of hemicellulose to **ethanol**. The PTS casAB gene from K. oxytoca M5A1 was used to transform **E. coli**, eliminating the need for additional beta-galactosidase (EC-3.2.1.23) during the simultaneous **saccharification** and **fermentation** of cellulose. To further reduce the need for cellulases (EC-3.2.1.4), plasmids containing the **celZ** gene from E. chrysanthemi P86021 and 'out' genes for secretion from E. chrysanthemi EC16, were added. The **CelZ** cellulase (EC-3.2.1.4) can degrade amorphous cellulose between pH 5.0 and 7.5 and should be compatible with bacterium or fungus cellulases. The best construct secreted over 25,000 U/l **endoglucanase** and may eliminate the need for supplemental **endoglucanase** during **saccharification** and **fermentation** of sucrose. (0 ref)

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
ACCESSION NUMBER: 1999:515364 CAPLUS
DOCUMENT NUMBER: 131:242039
TITLE: Engineering **endoglucanase**-secreting strains of ethanologenic **Klebsiella** oxytoca P2
AUTHOR(S): Zhou, S.; Ingram, LO
CORPORATE SOURCE: Institute of Food and Agricultural Sciences, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Journal of Industrial Microbiology & Biotechnology (1999), 22(6), 600-607
CODEN: JIMBFL; ISSN: 1367-5435
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant **Klebsiella** oxytoca P2 was developed as a biocatalyst for the simultaneous **saccharification** and **fermn**. (SSF) of cellulose by chromosomally integrating Zymomonas mobilis genes (pdc, adhB) encoding the **ethanol** pathway. This strain contains the native ability to transport and metabolize cellobiose, eliminating the need to supplement with .beta.-glucosidase during SSF. To increase the utility of this biocatalyst, we have now chromosomally integrated the **celZ** gene encoding the primary **endoglucanase** from Erwinia chrysanthemi. This gene was expressed at high levels by replacing the native promoter with a surrogate promoter derived from Z. mobilis DNA. With the addn. of out genes encoding the type II protein secretion system from E. chrysanthemi, over half of the active **endoglucanase** (EGZ) was secreted into the extracellular environment. The two most active strains, SZ2(pCPP2006) and SZ6(pCPP2006), produced approx. 24 000 IU L-1 of CMCase activity, equiv. to 5% of total cellular protein. Recombinant EGZ partially depolymd. acid-swollen cellulose and allowed the prodn. of small amts. of **ethanol** by SZ6(pCPP2006) without the addn. of fungal cellulase. However, addnl. **endoglucanase** activities will be required to complete the depolymn. of cellulose into small sol. products which can be efficiently metabolized to **ethanol**.

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 2000:842291 CAPLUS
DOCUMENT NUMBER: 134:16638
TITLE: Transgenic microorganisms capable of simultaneous **saccharification** of complex carbohydrate substrates and alcoholic **fermentation**
INVENTOR(S): Ingram, Lonnie O.; Zhou, Shengde
PATENT ASSIGNEE(S): The University of Florida Research Foundation, USA
SOURCE: PCT Int. Appl., 87 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000071729	A2	20001130	WO 2000-US14773	20000526
WO 2000071729	A3	20010830		
WO 2000071729	C2	20020704		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1185672	A2	20020313	EP 2000-941157	20000526
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 2000010963	A	20020319	BR 2000-10963	20000526

PRIORITY APPLN. INFO.: US 1999-136376P P 19990526
WO 2000-US14773 W 20000526

AB The invention provides recombinant host cells contg. at least one heterologous polynucleotide encoding a polysaccharase under the transcriptional control of a surrogate promoter capable of increasing the expression of the polysaccharase. In addn., the invention further provides such hosts with genes encoding secretory protein/s to facilitate the secretion of the expressed polysaccharase. Preferred hosts of the invention are ethanologenic and capable of carrying out simultaneous **saccharification fermn.** resulting in the prodn. of **ethanol** from complex cellulose substrates.

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:10662 CAPLUS
DOCUMENT NUMBER: 136:81966
TITLE: Synergistic hydrolysis of amorphous cellulose for **ethanol saccharification** and **fermentation** by recombinant **Klebsiella** oxytoca expressing two **endoglucanases** (**CelZ** and **CelY**) from Erwinia chrysanthemi
INVENTOR(S): Ingram, Lonnie O.; Zhou, Shengde
PATENT ASSIGNEE(S): University of Florida, USA
SOURCE: PCT Int. Appl., 132 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002000858	A2	20020103	WO 2001-US19690	20010619
WO 2002000858	A3	20020613		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002159990	A1	20021031	US 2001-885297	20010619

PRIORITY APPLN. INFO.: US 2000-214137P P 20000626
US 2000-219913P P 20000721

AB The present invention provides **endoglucanase** activities for carrying out the degrading of a complex sugar and more preferably, the use of **endoglucanase** activities in particular ratios for optimal degrading of a complex sugar. In addn., the invention provides recombinant host cells engineered for optimal expression and secretion of **endoglucanase** activities suitable for degrading complex sugars. Specifically exemplified are recombinant enteric bacteria, Escherichia and **Klebsiella**, which express an **endoglucanase** under the transcriptional control of a surrogate promoter for optimal expression. In addn., also exemplified is a recombinant enteric bacterium that expresses two different **endoglucanases celY** and **celZ**, where each is under the transcriptional control of a surrogate promoter for optimal expression in a particular ratio. The invention provides for the further modification of these hosts to include a secretory protein/s that allow for the increased prodn. and/or secretion of the **endoglucanases** from the cell. In a preferred embodiment, the invention provides for the further modification of these hosts to include exogenous ethanologenic genes derived from an efficient **ethanol** producer, such as Zymomonas mobilis. A deriv. of **Klebsiella** oxytoca M5A1 contg. chromosomally integrated genes for **ethanol** prodn. from Zymomonas mobilis (pdc, adhB) and **endoglucanase** genes from Erwinia chrysanthemi (**celY**, **celZ**) produced over 20 000 U **endoglucanase** 1-1 activity during **fermn**. In combination with the native ability to metabolize cellobiose and cellotriose, this strain was able to ferment amorphous cellulose to **ethanol** (58-76% of theor. yield) without the addn. of cellulase enzymes from other organisms. Erwinia chrysanthemi produces a battery of hydrolases and lyases which are very effective in the maceration of plant cell walls. In summary, these results using,

e.g., K. oxytoca strain SZ21, demonstrate an advancement toward the goal of producing sufficient cellulase enzymes for the direct bioconversion of cellobiosides and amorphous cellulose to **ethanol** without the addn. of supplemental enzymes. **Endoglucanase** levels produced by this strain are over 10-fold over those previously reported for engineered strains of yeast and other bacteria during **ethanol fermn** . (Brestic-Goachet et al. 1989, Cho et al. 1999, Cho & Yoo 1999, Misawa et al. 1988, Su et al. 1993, Van Rensburg et al. 1996,1998).

L10 ANSWER 2 OF 9 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09391 BIOTECHDS
TITLE: New composition comprising first and second **endoglucanase** having first and second degrading activity, useful for degrading complex sugar, especially oligosaccharide, into smaller sugar moieties; plasmid-mediated recombinant enzyme gene transfer and expression in **Klebsiella** oxytoca and Escherichia **coli** for **ethanol** production
AUTHOR: INGRAM L O; ZHOU S
PATENT ASSIGNEE: UNIV FLORIDA
PATENT INFO: WO 2002000858 3 Jan 2002
APPLICATION INFO: WO 2000-US19690 26 Jun 2000
PRIORITY INFO: US 2000-219913 21 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-164441 [21]
AB DERWENT ABSTRACT: NOVELTY - A new composition for degrading an oligosaccharide comprising a first and second **endoglucanase** having a first and second degrading activity, respectively, where the first and second degrading activities are present in a ratio such that the degradation of the oligosaccharide by the first and second **endoglucanases** is synergized. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) degrading or enhancing the degradation an oligosaccharide by contacting an oligosaccharide with a first and second **endoglucanase** having a first and second **endoglucanase** degrading activity, respectively, where the first and second degrading activities are present in a ratio where degradation of the oligosaccharide by the **endoglucanases** is synergized; (2) a recombinant host cell suitable for degrading an oligosaccharide; (3) enhancing the degradation of an oligosaccharide; (4) making a recombinant host cell suitable for degrading an oligosaccharide; (5) making a recombinant host cell integrant by introducing into the host cell a vector comprising the polynucleotide sequence of pLOI2352 (consisting of 11772 base pairs (bp) fully defined in the specification), and identifying a host cell having the vector stably integrated; (6) expressing an **endoglucanase** in a host cell by introducing into the host cell a vector comprising the polynucleotide sequence of pLOI2306 (consisting of 11544 bp give in the specification), and identifying a host cell expressing the **endoglucanase**; (7) producing **ethanol** from an oligosaccharide source; (8) a vector comprising the polynucleotide sequence of a plasmid or its fragment selected from pLOI2311, pLOI1620, pLOI2316, pLOI2317, pLOI2318, pLOI2319, pLOI2320, pLOI2323, pLOI2342, pLOI2348, pLOI2349, pLOI2350, pLOI2352, pLOI2353, pLOI2354, pLOI2355, pLOI2356, pLOI2357, pLOI2358, and pLO2359; (9) a host cell comprising a vector of (8); (10) an enzyme extract derived from the host cell; and (11) the recombinant host strain of **Klebsiella** oxytoca strains P2 (pCPP2006), SZ6 (pCPP2006), SZ21 (pCPP2006), and SZ22 (pCPP2006), represented by a deposit with the American Type Culture Collection (number not specified). BIOTECHNOLOGY - Preferred Composition: The first **endoglucanase** and/or second **endoglucanase**, are derived from a cell extract. The cell extract is derived from a bacterial cell, which has been recombinantly engineered to express the first and /or second **endoglucanase**. The bacterial cell is selected from family Enterobacteriaceae, particularly Escherichia or

Klebsiella. The cell extract comprises a first **endoglucanase** encoded by **celZ**, and a second **endoglucanase** encoded by **celY**, where **celZ** and **celY** are derived from Erwinia. The first **endoglucanase** is EGZ and the second **endoglucanase** is EGY. The ratio ranges from 9:1 to 19:1. The first and/or second **endoglucanase**, are purified. Degradation is synergized by a factor ranging from 1.1-2.0, preferably 1.8. The composition further comprises an additional enzyme selected from **endoglucanase**, exoglucanase, cellobiohydrolase, beta-glucosidase, endo-1,4-beta-xylanase, alpha-xylosidase, alpha-glucuronidase, alpha-L-arabinofuranosidase, acetylesterase, acetylxylanesterase, alpha-amylase, beta-amylase, glucoamylase, pullulanase, beta-glucanase, hemicellulase, arabinosidase, mannanase, pectin hydrolase, pectate lyase, or their combinations. Glucanase is derived from a fungus, preferably from T. longibranchiatum. The additional enzyme is an ethanologenic enzyme selected from pyruvate decarboxylase and alcohol dehydrogenase. The first **endoglucanase** and the second **endoglucanase** are packaged separately. The composition is used for simultaneous **saccharification** and **fermentation**. The oligosaccharide is selected from cellooligosaccharide, lignocellulose, hemicellulose, cellulose, pectin, and their combinations. Preferred Method: In enhancing oligosaccharide degradation, the oligosaccharide is contacted with the first and the second **endoglucanase** in any order or concurrently. The method is conducted in an aqueous solution. Degradation of an oligosaccharide is accompanied by a change in viscosity, preferably a reduction in viscosity by at least 5, 10, 20, 50, 100, 500, or 1000 centopoise. The oligosaccharide is cellulose from paper, pulp, or plant fiber. Enhancing the degradation of an oligosaccharide comprises: contacting an oligosaccharide with a host cell comprising a first and second heterologous polynucleotide segment encoding a first and second **endoglucanase** having a first and second degrading activity, respectively, where each segment is under the transcriptional control of a surrogate promoter, where the first **endoglucanase** and the second **endoglucanase** are expressed so that the first and the second degrading activities are present in a ratio such that oligosaccharide degradation by the 2 **endoglucanases** is synergized and enhanced. The method is conducted an aqueous solution, and the oligosaccharide is selected from of cellooligosaccharide, lignocellulose, hernicellulose, cellulose, pectin, and their combinations A recombinant cell for degrading an oligosaccharide can be prepared by introducing into the host cell a first heterologous polynucleotide segment encoding a first and second **endoglucanase** having a first and second degrading activity, respectively, where each segment is under the transcriptional control of a surrogate promoter, and the first and second **endoglucanase** activities are expressed such that oligosaccharide degradation by the first and second **endoglucanases** is synergized. The surrogate promoter of the first and/or second heterologous polynucleotide segment, comprises a polynucleotide fragment derived from Zymomonas mobilis. Producing **ethanol** from an oligosaccharide source comprises contacting the oligosaccharide source with an ethanologenic host cell comprising a first heterologous polynucleotide segment encoding a first and second **endoglucanase** having a first and second degrading activity, respectively, where each segment is under the transcriptional control of a surrogate promoter, and the first and second **endoglucanase** activities are expressed such that oligosaccharide degradation by the first and second **endoglucanases** is synergized resulting in a degraded oligosaccharide that is fermented into **ethanol**. The method is conducted in an aqueous solution, and the oligosaccharide is selected from of cellooligosaccharide, lignocellulose, hemicellulose, cellulose, and/or pectin. The heterologous polynucleotide segment is, or derived from, of pLOI2352. The surrogate promoter of the first and/or second polynucleotide segment comprises a polynucleotide fragment derived

from Zymomonas mobilis. Preferred Recombinant Host Cell: The recombinant host cell for degrading an oligosaccharide comprises: (a) a first heterologous polynucleotide segment encoding a first **endoglucanase** having a first degrading activity, where the segment is under the transcriptional control of a surrogate promoter; and (b) a second heterologous polynucleotide segment encoding a second **endoglucanase** having a second degrading activity, where the segment is under the transcriptional control of a surrogate promoter. The first **endoglucanase** and the second **endoglucanase** are expressed so that the first and second degrading activities are present in a ratio such that degradation of the oligosaccharide by the first and second **endoglucanases** is synergized. The secretory enzyme is a pul or out gene product. The host cell is ethanologenic, and is selected from **E. coli** KO4 (ATCC 55123), **E. coli** KO11 (ATCC 55124), **E. coli** KO12 (ATCC 55125), **E. coli** LY01 (ATCC 11303), and K. oxytoca P2

L7 ANSWER 11 OF 24 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 7
ACCESSION NUMBER: 1997:446412 CAPLUS
DOCUMENT NUMBER: 127:175452
TITLE: Production of recombinant bacterial **endoglucanase** as a co-product with ethanol during fermentation using derivatives of Escherichia coli KO11
AUTHOR(S): Wood, B. E.; Beall, D. S.; Ingram, L. O.
CORPORATE SOURCE: Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Biotechnology and Bioengineering (1997), 55(3), 547-555
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English
AB This study demonstrates a new approach to reduce the amt. of fungal cellulase required for the conversion of cellulose to EtOH. E. coli KO11, a biocatalyst developed for the fermn. of hemicellulose syrups, was used to produce recombinant **endoglucanase** as a co-product with EtOH. Seven different bacterial genes were expressed from plasmids in KO11. All produced cell-assocd. **endoglucanase** activity. KO11(pLOI1620) contg. **Erwinia chrysanthemi celZ** (EGZ) produced the highest activity, 3200 IU **endoglucanase**/L fermn. broth (assayed at pH 5.2 and 35.degree.). Recombinant EGZ was solubilized from harvested cells by treatment with dil. SDS (12.5 mg/mL, 10 min, 50.degree.) and tested in fermn. expts. with com. fungal cellulase (5 filter paper units/g cellulose) and purified cellulose (100 g/L). Using Klebsiella oxytoca P2 as the biocatalyst, fermns. supplemented with EGZ as a detergent-lysate of KO11(pLOI1620) produced 14%-24% more EtOH than control fermns. supplemented with a detergent lysate of KO11(pUC18). These results demonstrate that recombinant bacterial **endoglucanase** can function with fungal cellulase to increase EtOH yield during the simultaneous saccharification and fermn. of cellulose.

=> d 17 ibib ab 20-24

L7 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 15
ACCESSION NUMBER: 1987:80733 CAPLUS
DOCUMENT NUMBER: 106:80733
TITLE: Characterization of a new **endoglucanase** from **Erwinia chrysanthemi**
AUTHOR(S): Boyer, Marie Helene; Cami, Brigitte; Chambost, Jean Pierre; Magnan, Mireille; Cattaneo, Jeanne
CORPORATE SOURCE: Lab. Chim. Bact., Cent. Natl. Rech. Sci., Marseille, F-13009, Fr.
SOURCE: European Journal of Biochemistry (1987), 162(2), 311-16
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The structural gene coding for a new endo-.beta.-1,4-glucanase (cellulase) of E. **chrysanthemi** strain 3665, previously identified in a cosmid library, was subcloned into pUC18. The gene was expressed from a 1.9 .times. 103-base-pair insert and its direction of transcription was detd. The properties of the gene product purified from cell-free exts. of Escherichia coli were studied. The purified protein had an **endoglucanase** activity but was significantly different from the major **endoglucanase** Z secreted by E. **chrysanthemi** strain 3665. The new enzyme was designated **endoglucanase** Y and the related gene **celY**. In E. coli, most of the **endoglucanase** activity was found in the periplasmic space.

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Search Results -

Terms	Documents
endoglucanase WITH (EGZ or EGY)	2

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L1

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DATE: Wednesday, December 11, 2002 Printable Copy Create Case

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DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L1	endoglucanase WITH (EGZ or EGY)	2	L1

END OF SEARCH HISTORY

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L1: Entry 2 of 2 File: USPT May 9, 2000

DOCUMENT-IDENTIFIER: US 6060274 A
TITLE: Extracellular expression of cellulose binding domains (CBD) using Bacillus

Brief Summary Text (39):
Several CBD's have been expressed in E. coli, however, none has ever been reported expressed and secreted from Bacillus sp. E. coli as an expression host for heterologues proteins has several advantages over Bacillus spp., firstly because E. coli has a periplasmic space where proper folding of heterologues expressed genes are possible (for review see for example Hockney, R. C. (1994). Especially the oxidizing potential and the existence of disulfide oxidoreductases in the periplasma is necessary when expressing proteins with a functionality dependent on properly arranged disulfide bridges (Emmanuel Brun et al. (1995). Overproduction, purification and characterization of the cellulose binding domain of the Erwinia chrysanthemi secreted endoglucanase EGZ is disclosed in Eur. J. Biochem 231, 142-148, and Ong et al., (1993). Further examples of CBDs with disulfide bonds are: the N-terminal CBD of CelB from Pseudomonas fluorescens subsp cellulosa (NCIMB 10462) (see the alignment in Tomme P. et al., op. cit., and the N-terminal CBD of CenA from Cellulomonas fimi (ATCC 484), N. R. Gilkes et al. (1991).

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Search Results - Record(s) 1 through 2 of 2 returned.

☐ 1. Document ID: US 20020159990 A1

L1: Entry 1 of 2 File: PGPB Oct 31, 2002

PGPUB-DOCUMENT-NUMBER: 20020159990
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020159990 A1

TITLE: Methods and compositions for simultaneous saccharification and fermentation

PUBLICATION-DATE: October 31, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ingram, Lonnie O?apos;Neal	Gainesville	FL	US	
Zhou, Shengde	Gainesville	FL	US	

US-CL-CURRENT: 424/94.61; 435/105, 435/161

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6060274 A

L1: Entry 2 of 2 File: USPT May 9, 2000

US-PAT-NO: 6060274
DOCUMENT-IDENTIFIER: US 6060274 A

TITLE: Extracellular expression of cellulose binding domains (CBD) using Bacillus

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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Terms	Documents
endoglucanase WITH (EGZ or EGY)	2

Display Format: - Change Format

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L7 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 10

ACCESSION NUMBER: 1993:33853 CAPLUS
DOCUMENT NUMBER: 118:33853
TITLE: Sequence analysis of the cellulase-encoding **celY** gene of **Erwinia chrysanthemi**: a possible case of interspecies gene transfer
AUTHOR(S): Guiseppi, Annick; Aymeric, Jean Luc; Cami, Brigitte; Barras, Frederic; Creuzet, Nicole
CORPORATE SOURCE: Lab. Chim. Bact., CNRS, Marseille, 13277, Fr.
SOURCE: Gene (1991), 106(1), 109-14
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The E. **chrysanthemi** (strain 3937) **celY** gene encoding the minor **endoglucanase** (EGY) was sequenced. The anal. of the upstream region allowed identification of an in vivo active promoter recognized by the NtrA (.sigma.54) holoenzyme. There was no similarity between the predicted amino acid (aa) sequences of EGY and either the Er. **chrysanthemi** major **endoglucanase**, EGZ, or the Er. carotovora CelS **endoglucanase**. In contrast, a very high level of identity, both at the nucleotide and the predicted aa levels, was found between **celY** and an EG-encoding gene from Cellulomonas uda, a gram+ bacterium taxonomically distant from Er. **chrysanthemi**. By comparing the molar G + C% of the cellulase-encoding genes and that of Er. **chrysanthemi** and C. uda chromosomal DNAs, it is proposed that **celY** was transferred from Er. **chrysanthemi** to C. uda.

L7 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 12
ACCESSION NUMBER: 1990:437000 CAPLUS
DOCUMENT NUMBER: 113:37000
TITLE: Homology between **endoglucanase** Z of **Erwinia chrysanthemi** and **endoglucanases** of Bacillus subtilis and alkalophilic Bacillus
AUTHOR(S): Guiseppi, A.; Cami, B.; Aymeric, J. L.; Ball, G.; Creuzet, N.
CORPORATE SOURCE: Lab. Chim. Bacterienne, CNRS, Marseille, 13277/9, Fr.
SOURCE: Molecular Microbiology (1988), 2(1), 159-64
CODEN: MOMIEE; ISSN: 0950-382X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Nucleotide sequencing of the **celZ** gene encoding the extracellular **endoglucanase** Z of E. **chrysanthemi** indicated the presence of an open reading frame encoding 428 amino acids. The mature protein appeared to be extended by a signal peptide of 43 amino acids; this sequence is unusually long and pos. charged (+5). It was shown to function as a signal peptide by fusing it to a truncated phoA gene encoding Escherichia coli alk. phosphatase. Comparison of the encoded sequence with those of the **endoglucanases** of B. subtilis and alkalophilic Bacillus revealed the existence of a region of extensive homol. occurring in all three proteins at about the same distance from the NH2-terminal end. These regions may be involved in substrate binding and/or catalytic sites.

L7 ANSWER 9 OF 24 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
ACCESSION NUMBER: 1999:515364 CAPLUS
DOCUMENT NUMBER: 131:242039
TITLE: Engineering **endoglucanase**-secreting strains of ethanologenic Klebsiella oxytoca P2
AUTHOR(S): Zhou, S.; Ingram, LO
CORPORATE SOURCE: Institute of Food and Agricultural Sciences, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA
SOURCE: Journal of Industrial Microbiology & Biotechnology (1999), 22(6), 600-607
CODEN: JIMBFL; ISSN: 1367-5435
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant Klebsiella oxytoca P2 was developed as a biocatalyst for the simultaneous saccharification and fermn. (SSF) of cellulose by chromosomally integrating Zymomonas mobilis genes (pdc, adhB) encoding the ethanol pathway. This strain contains the native ability to transport and metabolize cellobiose, eliminating the need to supplement with .beta.-glucosidase during SSF. To increase the utility of this biocatalyst, we have now chromosomally integrated the **celZ** gene encoding the primary **endoglucanase** from **Erwinia chrysanthemi**. This gene was expressed at high levels by replacing the native promoter with a surrogate promoter derived from Z. mobilis DNA. With the addn. of out genes encoding the type II protein secretion system from E. **chrysanthemi**, over half of the active **endoglucanase** (EGZ) was secreted into the extracellular environment. The two most active strains, SZ2(pCPP2006) and SZ6(pCPP2006), produced approx. 24 000 IU L-1 of CMCase activity, equiv. to 5% of total cellular protein. Recombinant EGZ partially depolymd. acid-swollen cellulose and allowed the prodn. of small amts. of ethanol by SZ6(pCPP2006) without the addn. of fungal cellulase. However, addnl. **endoglucanase** activities will be required to complete the depolymn. of cellulose into small sol. products which can be efficiently metabolized to ethanol.